Final Report of Prof. Luc Montagnier and Prof. Vittorio Colizzi to Libyan Arab Jamahiriya on the Nosocomial HIV infection at the Al-Fateh Hospital, Benghazi, Libya

(Paris, 7 April 2003)

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1. The scientific international consultants

1.1 Appointment of the scientific international consultants

Prof. Luc Montagnier (Paris, France) and Prof. Vittorio Colizzi (Rome, Italy) have been appointed as international scientific consultants by the Secretary of the Libyan Arab Jamahiriya (see enclosed letters by the Assistant Secretary of the General Peoples Committee Mr. Ammar Mabrok Ltaif). Official letters have been sent also to all the foreign European Hospitals that have received the Libyan children for clinical management and therapeutic treatment in Italy (Milan and Rome), in Austria (Vienna), in France (Paris) and in Switzerland (Geneva and Lausanne) asking full cooperation for the required enquired on the nosocomial HIV infection at the Children Hospital Al-Fateh in Benghazi, Libya.

1.2 Meeting on the 16th of May 2002 at the UNESCO building, c/o the World Foundation for AIDS Research and Prevention.

Ambassador Mohamed Ahmed Alaswad (Permanent delegation of Libya at UNESCO), Prof. Luc Montagnier, Prof. Vittorio Colizzi, Dr. Piero Vagliani, Dr. Mustafa Eteer and Dr. Muktar Soussi were present. The following advancement of the activity has been summarised by Prof. Montagnier:

2 Appointment of Prof. Montagnier and Prof. Colizzi as scientific consultants;
3 Letters to all hospitals involved in the recovering the Libyan children asking cooperation for the scientific consultants;
4 Analysis of the Report on the WHO mission carried out in Benghazi on the December 1998;
5 Analysis of the 2 published papers reporting sequences of the HIV and HCV isolates from the infected children (see below).

After a common discussion, the following decisions of this Meeting at UNESCO were undertaken:
- organise a site visit for Prof. Colizzi at the Benghazi Hospital to have access to all the files regarding the infected subjects available at the Hospital and to collect all the samples, if available;
- collect all data and samples, if available, from other hospitals involving those children that were admitted and treated previously;
- carry out sequences of HIV and HCV isolates from other samples for children and adults infected at the Benghazi Hospital and from other local communities (maternity, drug abusers). The sequences will be performed in independent laboratories;
- perform a Scientific Report about all data collected and suitable considerations on the infection within the end of July.


This report describes the visit performed by the WHO team (Dr. P.N. Shrestha, dr. A. Eleftherious and Dr. V. Giacomet) to Tripoli, Sirte and Benghazi the 28 December 1998-11 January 1999. The topic outlined by the Report are the following: HIV/AIDS Situation at the Al-Fateh Hospital, Infection Control, Clinical management, Counselling, Laboratory facilities, Blood safety, Health education, Sexual Transmitted Diseases, Perinatal transmission, Injection drug use, Surveillance, Programme Management, Recommendations.

Analysis of the Report on the WHO mission carried out in Benghazi on the December 1998-January 1999 has been summarised. This Report strongly suggests that the nosocomial HIV infections at the Al-Fateh Hospital were caused by multiple sources of infections. Moreover, the WHO team notes the lack of required supplies and equipment such as sharp container, sterilizer, incinerator, protective gloves, etc. Considering the lack of knowledge, skill and attitude of a number of staff, the WHO team provided appropriate guidelines. Dr. Stefano Lazzari, Coordinator ISR/CSR/EDC, on the 4 October 1999 sent a Memorandum to Dr. Jihane Tawilah regarding the two travel reports in Benghazi. The WHO has noted several similarities with previously documented outbreaks in children such as in Elista, USSR in 1988 and in Romania in 1990. In particular, the practice of using in dwelling intravenous catheters for injections in hospitalized children and sharing the same syringes without appropriate sterilization, would appear to be possible causes of the outbreak in Benghazi.

1.3. Visit of Prof. V. Colizzi at the Al-Fateh Hospital in Benghazi (14-19 June 2002).

According to the official letter of the Libyan Government in which Prof. Luc Montagnier and Prof. Vittorio Colizzi have been appointed as international scientific consultants, and following the indications discussed during the Meeting held in Paris at the UNESCO Headquarter with the
presence of the Libyan Ambassador M.M. Alaswad, Dr. M. Eteer and Dr. M. Soussi, the aims of the visit are the following:

- Site visit at the Centre for Immunodepressed Children and at the Al-Fateh Children Hospital;
- Collection of all clinical data regarding the infected children, including the list of the children sent in foreign hospitals for care and antiretroviral treatments;
- Collection of samples (plasma) eventually still present in the Al-Fateh Hospital;
- Collection of plasmas from adult seropositive individuals from the Benghazi area.

The visit started with the Centre for Immunodepressed Children (HIV-infected children), established one year ago to follow the 404 children infected at the Al-Fateh Children Hospital. Dr. Amina Saleh Abusedra (tel. 2229799; E-mail dramina2002@hotmail.com) has been introduced as the Head of the Laboratory, while Dr. Ali is one of the medical staff at the clinical department. The Centre has been extensively visited, and a general discussion has been carried out with Dr. Amina Abusedra. In particular, evidence has been obtained that the majority of HIV infected children are followed on a regular basis.

However, from the clinical point of view, immunological studies should be performed in addition to the CD4/CD8 quantification. Moreover, considering the toxic effect of the drugs on the metabolism alteration of the myocardium, cardiology analysis has to be included at least once per year. The Laboratory of the Centre is well equipped for routine virological, immunological and clinical analyses, but the Tissue Culture Facility is not available, and the present flow cytometry equipment is not sufficient to analyse also the functional activity of the immune system. Finally, the mutations of HIV sequences associated to the appearance of drug resistance have to be evaluated; a sequencer apparatus has to be set up at the Centre. The possibility to introduce Structural Intermittent Therapy should be evaluated, also considering that some children and their mother are already under antiretroviral treatment, while others some will be, soon or later, treated.

Moreover, Dr. Amina Abusedra also reports the recent results of an HCV screening of the HIV infected children followed at the Centre: new 50 cases of HCV seropositivity were found with a consistent increase in the percentage of HCV infection in the cohort of HIV infected children. No evidence was available as to whether these recent HCV positivity should be considered new infections or undetectable old infections. In the first case, some procedure may still be improved in Benghazi hospital. In the second case, the high percentage of HCV infection (average 75%) in the HIV-positive paediatric cohort would strongly indicate a massive nosocomial coinfection at that time (1998-99). An epidemiological investigation is urgently needed to solve this aspect (i.e. a
comparison between the percentage of HCV seropositivity in the children population which never attended a hospital with that of hospitalised children for one year and now released).

Two HIV infected nurses (M.I. and S.M) have been interviewed with the help of Dr. G. Gemial, both of them worked at the Al-Fateh Children Hospital at the time of the nosocomial HIV infections. M.I. in the Gastroenterology and Isolation, while S.M. in the Paediatric Ward A. From the sequencing data in press on AIDS & Human Retroviruses, the HIV isolated from the two nurses have very similar sequences when compared to the DNA sequences of the HIV isolates obtained from 23 infected children and from 15 infected mothers. The nurse M.I. referred that she had self injection while she was transferring the patient’s blood from the syringe to the test tube. The nurse S.M. received three infusions of dextrose due to a sincopy attack.

Finally, during the visit at the Centre for Immunodepressed Children, several mothers and fathers asked to discuss several points, such as the therapy of their children, the possibility of viral transmission from infected mother to father by sexual intercourse, etc. The general feeling is that a lack of communication occurs between families and the Centre. The communication and specific counselling is a top priority in such cases of nosocomial HIV infection, particularly when so high a number of persons are involved.

The visit has been also carried out at the Al-Fateh Children Hospital. Here the complete list of HIV infected children has been shown by the Head of the Computer Department in the Excel software Programme, including the specification of European hospitalisations for each child. Moreover, the list includes other new 9 cases recently identified (2001, 2002), that has been admitted in the Hospital at the time of the outbreak. Other files contained all data about the age, nationality, sex, date of birth, date of diagnosis, date of admission, etc. A specific file contains the list of the HCV seropositive children attending the Hospital. In particular, it is relevant to know the general percentage of HCV recording in the hospital data, and whether other cases of HIV infected children have been reported in 2000, 2001, 2002. All these informations are present in the Excel Data Bank of the Al-Fateh Children Hospital. The digital form (Excel Programme) of the lists may be obtained on a diskette, upon written request of the Director of the Hospital.

To obtain all clinical and laboratory data from the Al-Fateh Hospital, a written authorisation has been required from Dr. Awad Abudejaja, President of the Research and Screening Programme (see enclosed letter in Arab). Dr. Awad Abudejaja was met by Dr Colizzi and the written authorisation obtained. Unfortunately, the engineering responsible for the data management was not any more
there, and no data were collected. For this reason, in the absence of any list of HIV infected children, the visit was stopped.

Monday 17 June, upon request of Dr. Mustafa Etheer from Tripoli, 3 IBM 1.4 MB diskettes containing Excel and Window data arrived from the Al-Fateh Hospital carried out personally by the President of the Family Association. All diskettes contained unprotected files, except two which were opened after the password was provided by telephone from the Head of the Computer Department of the Al-Fateh Hospital on Tuesday 18 June 2002. All diskettes were printed and the translation of the children names from Arab to English was initiated.

Regarding the collection of clinical samples (sera or plasma), the medical staff at the Al-Fateh Children Hospital has been interviewed to ascertain whether original clinical samples are still present and available in the Hospital (at −20°C). Unfortunately there was a general consensus that clinical samples were no more available.

1.4. Obtaining frozen lymphocytes from children which were hospitalised in Geneva (Switzerland, Hôpital Cantonal) and in Rome (Italy Hospital Bambin Gesù).

In order to further analyze HIV sequences which were present in hospitalised children, we sent letters of request to Dr. Luc Perrin (Hôpital Cantonal de Genève), Dr. Christine Rouzioux (Hôpital Necker Paris), Dr. Paolo Rossi (Hospitalo Bambin Gesù) and Dr. Giuseppe Ippolito (Hospital L. Spallanzani) who had kept frozen some lymphocyte samples of the children formerly hospitalized in these hospitals.

We thus received from Dr Perrin, 23 samples of frozen lymphocytes (at -80°C) corresponding to 23 children who where hospitalized in Geneva in 2000. Sequences were sent for molecular analysis in an independent laboratory (Vironix/Institut A. Frappier, Montreal (see results below).

Other samples of frozen plasma and lymphocytes from 100 children were obtained by the Hospital Bambin Gesù and sent for molecular analysis in an independent laboratory in Padua (see results below).

Considering the absence of relevant samples (i.e. samples from children probably infected in 1997, or after the leave of the Bulgarian staff from the Benghazi Hospital, new samples from selected children are considered necessary.
1.5. Visit of Prof. V. Colizzi and his two collaborators, Dr. Guido Castelli Gattinara and Dr. Massimo Amicosante at the Centre for Immunodepressed Children in Benghazi (28 January-7 February 2003).

The mission has been undertaken for 10 days, starting the 28 January and finishing the 7 of February 2003. The mission was carried out for the first 7 days in Benghazi and for the last 3 days in Tripoli. Dr. Mustafà Eteer, director of the Centre of the Infection Disease Control Africa, CIDCA, was the Libyan doctor taking care of the mission both in Benghazi and in Tripoli.

This Mission had two distinct objectives, as requested by the Libyan authorities during the first meeting in Tripoli on the 20-21 March 2002. The first was to finalize the investigation on the origin of the nosocomial infection at the Benghazi Children Hospital. The second objective was related to the clinical and laboratory follow up of the children, and to the identification of all measures required to set up the best clinical and laboratory procedures.

During this 7 days visit at the Benghazi Centre, 140 children have been visited by the Libyan and Italian doctors. Dr. Guido Castelli Gattinara was always present and personally checked all children, discussing all clinical charts, speaking with all parents and suggesting the right therapy to the Libyan doctors. A consensus format for the clinical check and blood work in Arabic language have been signed by the parents and then sent to the Italian General Consulate in Benghazi for the official translation.

From most of the HIV-infected children it has been collected 5-7 ml of peripheral blood. The plasma have been stored, the mononuclear cells isolated by Ficoll-Hypaque gradient and frozen at –80°C. Due to the fact that there is not dry ice or liquid nitrogen transport system in Benghazi, all biological samples containing cells have been left in the freezer at the Centre. A few blood samples collected the last days have been transported at room temperature and immediately analysed in Rome. All plasma have been used for the viral load detection (Benghazi Centre), for the genetic analyses for drug resistance (Rome) and for the understanding of the origin of the nosocomial infection (Rome and Paris).

The clinical data present in the patient’s charts at the Centre have been matched with data present in the original diskette received by Benghazi Children Hospital in June 2002. Moreover, more than 100 original clinical charts have been obtained by the Benghazi Children Hospital, as
requested officially to Dr. Mustafà Eteer. A complete new version of the data have been given to Dr. M. Eteer with a formal requirement to fill all lacking data. Dr. Eteer had planned to send to Prof. Colizzi all requested data in 2-3 weeks, but this is still not arrived.

Prof. Vittorio Colizzi and Dr. Mustafa Eteer went then to Tripoli to collect the plasma flask supposed to be at the origin of the infection, as previously requested by a written letter by Prof. Montagnier. The bottle is stored at the Ministry of Justice, but after three days of waiting there was no possibility to have access to this bottle for HIV molecular analysis.

In these few days in Benghazi and Tripoli, the mission’s staff greatly appreciated at personal level the collaboration carried out by Dr. M. Eteer and by all the personnel of the Centre. However, in our opinion the actual standard of organisation and management of the Centre is not sufficient to give the best clinical care of these children. A specific Plan is necessary to make the organisation of the Centre adapted to the clinical care of children who acquired the HIV infection in the hospital (and then severely hit by some other diseases). As already discussed during the last meeting in Benghazi with all the medical staff of the Centre, there is a impressive absence of procedures, guidelines, clinical protocols, safety measures, training of personnel, psychological support, communication capacity, etc, etc.. Finally, there are too many shortages of drugs, equipment, materials and reagents which do not allow to perform the best clinical and laboratory practices on these children. A specific Laboratory for cellular immunology needs to be set up. Moreover, a specific budget should be provided also for tests (eg. genotyping drug resistance) not yet available in Libya, but strictly required for HIV-infected children which show clinical and laboratory evidence of genetic resistance to the antiretroviral treatment. Finally, an update to clinical and scientific international literatures is required for the medical staff operating at the Centre.

2. Scientific Publications on the Benghazi Nosocomial Infection

2.1. Nosocomial Outbreak of Multiple Bloodborne Viral Infections (S. Yerly et al., J.Inf. Dis. 2001)

The summary of the Publication is reported and the most relevant conclusions are outlined.

“In resource-limited countries, nosocomial transmission of bloodborne pathogens is a major public health concern. After a major outbreak of human immunodeficiency virus (HIV) infection in the average of 400 children in 1998 in Libya, we tested HIV, hepatitis C virus (HCV), and hepatitis
B virus (HBV) markers in 148 children and collected epidemiological data in a subgroup of 37 children and 46 parents. HIV infection was detected in all children but one, with HCV or HBV coinfection in 47% and 33%, respectively. Vertical transmission was ruled out by analysis of parent’s serology. The children visited the same hospital 1-6 times; at each visit, invasive procedures with potential blood transmission of virus were performed. HIV and HCV genotypic analysis identified a HIV monophyletic group, whereas 4 clusters of HCV sequences were identified. To our knowledge, this is the largest documented outbreak of nosocomial HIV transmission.”

The conclusions made by the Authors is that this outbreak is of nosocomial origin for the following facts:
- the monotypic characteristic of the HIV sequences, with very low interisolate variation;
- all children attended the same hospital and underwent invasive procedures;
- the serological data of a subgroup of parents exclude vertical transmission in all of the cases;
- there is a high incidence of concomitant HIV, HCV and even HBV infection, despite an active HBV vaccination program;
- some children were coinfect ed with HIV and HCV during the same invasive procedures.

Finally, for the Authors “the most likely scenario is that a first child was infected with HIV through contaminated injection material or through an unidentified vertical transmission. Ongoing acute primary HIV infection, with several children carrying very high viremia, then would have contributed to the explosive spread of HIV infection”.


The summary of the Publication is reported and the most relevant conclusions are outlined.

“A cluster of HIV-1 infected children has been identified in Libya, involving 402 children admitted to the El-Fath Children’s Hospital in Benghazi (BCH) during 1998 and 19 of their mothers. Nosocomial transmission has been identified as responsible for the spread of infection. Out of this group, 104 children and 19 adult women have been followed at the National Institute for Infectious Diseases “L. Spallanzani” in Rome, during one year. At BCH, all children, but one had received intravenous infusions but not blood or blood products. A single child received a blood transfusion in 1997 and the 17 infected mothers were never hospitalized in Benghazi. In addition, two nurses were diagnosed as HIV-1 infected. In 40 subjects out of this group HIV-1 gag, env and
pol fragments were amplified and sequenced. The phylogenetic analyses showed that a monophyletic recombinant HIV-1 form CRF02-AG was infecting all the HIV-1 seropositive patients admitted at BCH with no close similarities with the other CRF02-AG reported to GenBank. A different strain was found in the child infected with the blood transfusion. The data thus suggest a highly contagious nosocomial spread of HIV-1 infection and possibly transmission of the virus from child to mother during breastfeeding in connection with primary HIV-1 infection”.

The conclusions made by the Authors is that “the BCH hospital HIV-1 nosocomial outbreak in 1998 was caused by a single source of infection with a CRF02-AG recombinant strain with no close similarities to any of the strains reported to GenBank until now”. The Authors also comment that “it is not impossible that during the last decade the infection has been introduced in the country through immigrants from Central Africa, and thereafter spread in individual cases by sexual transmission or by contaminated blood transfusion”.

3. Clinical Data Analysis obtained in June 2002 and February 2003

3.1. Clinical Data obtained in June 2002

All clinical data have been obtained in digital form by the Computer Service of the Al-Fateh Hospital. The serial numbers are listed in the attached file Word-PC. A complete epidemiological analysis is still in progress, as several data are still lacking. However, a preliminary analysis of the data (admission and diagnosis, date of birth, Ward and Unit of the Hospital, etc.) allows to allocate the HIV-infected individuals in the following distinct categories

*Category A (n. 7 children)*

At least 7 children have been infected in the years 1994-1997, with no admission in the Hospital in the year 1998: (n. 308, 312°, 340, 350, 356, 373, 385).°no age


The comment is that the Ward B was already heavy contaminated in November 1997

*Category B (3 children)*
Children admitted and found seropositive after the 9 February 1999, date of departure of the Bulgarian staff.
N. 349, 376, 384°

The comment is that the infection was still active also in the absence of the Bulgarian staff.

**Category C (15 patients)**

At least 14 Children and 1 adult are either admitted in the Hospital in the first 2 months 1998 (January-February, before the arrival of the Bulgarian staff) or later with the date of seropositivity not compatible with the date of admission, i.e. they have been infected in the year 1997 or previously.

Those are N. 1, 31, 32, 73, 134, 143, 180, 211, 264, 326, 333, 334, 355, 389, 410

N. 1 ISO in 1997, N. 31 is already positive at the admission and has to be infected previously but there is no information on previous admission in the Hospital, N. 73 may have acquired the infection in Ward B in February 1998, N. 134 same situation like 73, 143 like 73 and 134, N. 180 like 73 and 134 and 143, N. 211 like 73 and 134 and 143 and 180, N. 264 like others but in Ward A, N. 326 like 73 and 134 and 143 and 180 and 211, N. 333 and 334 like others in Ward B at the same time, N. 355 like others but in Ward A and B, N. 389 may be the Libyan nurse which worked in Ward B and recognized to be punctured by the collected blood from patients, N. 410 in Ward C at the same time of the others but also in 1997.

**Category D (7 children)**

At least 7 HIV-infected children are shown to be seropositive less than 1 month after their last admission in 1998. However, the majority of the infected children have documented admission in the previous years, strongly indicating that the real year of infection is not 1998 but 1997 and even 1996, 1995, 1994.

N.6: recovered on August 1998 and was found seropositive less than one month later. This 1 year old child has been probably infected at birth in the Maternity Hospital, or during early vaccination. We need to know the seropositivity status of the mother.

N.9: recovered twice in 1997, only once in 1998 (august) and found seropositive after 1 month: very unlikely that a person 20 years old becomes seropositive 1 month after infection. More probable the infection happened in the year 1997 in Ward C.

N. 10 Child 11 years old admitted on 20 August and found seropositive few days later on the 7 of September: also this child could be infected previously in the Hospital or in other place.

N. 11. Child 7 year old as n. 10
N. 16: Child 7 year old as N. 10 and 11
N. 19 Child 9 year old as N. 10, 11, 16

For infections included in Categories A-B-C-D there is no evidence that correlate infections with the presence of the Bulgarian staff in the Al-Fateh Hospital (arrival: first week of March 1998; till 9 February 1999): total numbers are 32. But, more importantly, Categories A and C definitively prove that the HIV infection in the Al-Fateh Hospital was already active in 1997. The identity in the cluster of DNA sequences of the HIV in this nosocomial infections, published by the Swiss and by the Italian groups, strongly indicate that the infection already existed in 1997 and was capable to spread in 1998 and in 1999.

Category E (37 children)

Children seropositive more than 1 month but less than 3 months from the last admission in 1998. Some of them have several admissions in previous years, and the infections may be correlate to previous admissions, considering the time required for the appearance of seropositivity.

N. 8, 13, 15, 20, 26, 28, 29, 32, 36, 37, 40, 46, 47, 54, 55, 58, 63, 70, 71, 85, 88, 92, 93, 97, 109, 116, 122, 127, 129, 136, 139, 145, 147, 150, 151, 162, 325

Category F (59 patients)

No information available for the date of diagnosis or admission


Category G (282 patients)

Individuals which correlate the date of infection with the presence of Bulgarian staff, but for many of them there is evidence of previous admissions in the Hospital or professional activity in the Hospital (2 Libyan nurses), and of infected mothers.

N. 4, 5, 14, 18, 21, 22, 23, 24, 25, 30, 33, 35, 38, 39, 41, 43, 44, 45, 48, 49, 51, 52, 53, 56, 57, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 72, 74, 75, 78, 82, 84, 86, 87, 89,90, 91, 94, 96, 98, 99, 100, 102, 103, 104, 105, 106, 107, 108, 111, 112, 113, 114, 115, 117, 118, 120, 123, 124, 125, 126, 128, 130,
Infected Adults (>16)
9, 18, 69, 76, 152, 175, 205, 213, 321, 337, 354, 359, 361, 362, 379, 380, 382, 389, 396,


General comments about the data
All sera tested for HIV antibodies have been collected since the 10 of August 1998, although some children have been infected in the previous years.

3.2. Clinical Data obtained in February 2003

Newly discovered cases were admitted in the Centre of Immunodepressed Children in the last year, and a new digital file was then prepared by the Centre according all the patients (adults and children) attending the Centre. Of course this List is different from the List of the Benghazi Children Hospital given to us on the June 2002 above described.

Most of the newly discovered cases were admitted to the Benghazi Children Hospital Al Fateh during January 1998 and January 1999. Few cases of patients that are of particular interest for understanding the possible origin of infection were interviewed with the help of an Arab translator and Libyan doctors. In particular, three cases strongly suggest the vertical transmission of HIV may
also be a cause of HIV infection of children subsequently admitted at the Benghazi Children Hospital. Case 1: one child expired 4 month after his birth, and this is probably due to the HIV in utero infection from his seropositive mother. Case 2: a seropositive mother developed full AIDS disease and was then submitted to antiretroviral treatment at the Spallanzani Hospital already in the 2000, when her child was admitted in the Benghazi Children Hospital in the late 1998. Her infection was difficult to explain by a retro-infection from her baby (she stopped breast-feeding when the child was recovered at the Hospital) and the short period of time between exposure to her baby and the occurrence of full blown AIDS would indicate that this mother may have been infected before the birth of his child. Case 3: a seropositive woman who delivered her baby on the January 1999 was then admitted to the Hospital in march 1999, when the nosocomial infection was already under control. However, genetic analysis of the viral RNA from the plasma collected from this child (case 3) has provided direct evidence that this infection was also due to the same virus responsible for the nosocomial infection. *These and other cases which can also be found may suggest that a low level of vertical transmission and of naturally HIV-infected children was present in the Benghazi area in 1997.*

Moreover, it is of interest to present the case of a 15 year old girl which was infected at the Benghazi Children Hospital in January 1998 (then before the arrival of the Bulgarian staff). When interviewed in the presence of Libyan doctors she denied (and the father independently had confirmed her daughter) any further admission in the Hospital during the 1998 and 1999. This strongly indicates that the nosocomial infection was already present in January 1998. Moreover, genetic analysis of the viral RNA from the plasma collected from this girl has provided direct evidence that this infection was also due to the same virus responsible for the nosocomial infection. Later on Dr. Mustafà Eterr discussed this case with the doctor in charge of her at the Benghazi Children Hospital and they indicate the possibility that this girl was exposed to blood collection during the year, which is not recorded in her chart. This raises the question of the recording system of the Benghazi Children Hospital, as we found some cases (like two thalassemic patients) that were surely admitted at the Hospital monthly for their clinical status, but there is not record of their admissions.

The overall stratification of the data of admission and seropositivity and genetic analysis of the viral RNA (see below) from the plasma collected from children admitted the last time at the Al-Fateh Hospital in 1997 (April in one case, September in a other case, December in a third case) has provided direct evidence that this nosocomial infection was already present in April 1997, has reached his peak in the June-August 1998, then decreased at the end of 1998, but was still present in March 1999. This epidemiological analysis of data is compatible with a possible future
identification of new cases of children either admitted in the Hospital Al-Fateh or coming form vertical transmission of HIV infection, present in Libya at low level (see other cases in Tripoli).

4. Molecular analysis of samples

The co-operation with the Centre for Immunodepressed Children in Benghazi and with all parents which gave a written consensus, have allowed to obtain plasmas from infected children and their mothers to perform detailed genetic analysis. Moreover, old samples frozen in Geneve (Cantonal Hospital) and Rome (Hospital Bambin Gesù) were sequenced for comparison. Finally, samples from Ivory Coast were sequenced to compare with the variability of the A/G subtype found in Benghazi.

The specific aims of the molecular analysis are the following:
- to confirm the A/G subtyping of HIV-1 involved in the nosocomial infection;
- to compare the HIV RNA sequences of samples from children infected in 1997, 1998 and 1999, measuring the number and the percentage of mutation and then variability of the HIV strain responsible for the nosocomial infection;
- to compare the level of variability of the A/G HIV strain of Benghazi with the variability of other HIV A/G strains collected in Ivory Coast;
- to associate HIV RNA sequences to possible mechanisms of pathogenesis and virulence.

The A/G subtype responsible for the nosocomial infection was independently confirmed both in Rome (V.V.) and in Paris (L.M.). In particular, the env gene from children samples has been sequenced after amplification with specific primers in Montreal, while the Gag gene has been sequenced in Rome (amplification) and in Padua (automatic sequencing) from samples of Benghazi and Ivory Coast. The samples of Ivory Coast have been sequenced considering the unavailability of other samples from Libya (out of Al-Fateh Children Hospital) and the high percentage of the A/G HIV-1 subtype in Subsharian Africa, including Ivory Coast. Few A/G sequenced are actually present in the Gene Bank and detailed comparison required very similar methods and amplification gene regions.

Previous genetic analysis on the env gene has given the information of the general variability of this gene which contains highly variable regions. However, all Benghazi samples sequenced show
very low genetic variability (less than 10%), suggesting a common origin of the nosocomial infection.

For the analysis of the gag fragment, after a first amplification of 1892bp of the viral RNA containing all the full length GAG product, 4 different sequences were performed for each samples in order to cover all the area of p17 and p24 of GAG. Sequences were made at the Bio-Molecular-Research centre of the University of Padua (http://bmr.cribi.unipd.it/) in blindly on coded samples. Electrophorogrammes within the sequences were electronically sent back to the Laboratory of Molecular Pathology at the University of Rome “Tor Vergata” for further analysis. The full-length gag sequence of each sample was recomposed by using the following programmes: Chromas-2 (www.technelysium.com.au) and GeneRunner (Hasting Software, Inc.). A fragment of 790bps spanning the area of p17 and p24 of gag was then analysed for variability between the different samples by using the ClustalW facility at the Europen Bioinformatic Institute (www.ebi.ac.uk). Subtyping analysis of the obtained sequences was made with the HIV-1 Subtyping Tool at the (http://www.ncbi.nlm.nih.gov/retroviruses/subtype/makepage.cgi?page =sub&type=0), by using the following reference sequences: 328902|A, 2745742|A, 3808250|A, 2570232|A, 1906382|B, 1465777|B, 328440|B, 328565|B, 1353860|C, 2194183|C, 3252927|C, 4324723|C, 328154|D, 329377|D, 326675|D, 2570307|D, 2570289|D, 3114544|F1, 5668938|F1, 6090965|F1, 6093151|F1, 6093141|F2, 6093146|F2, 2570325|G, 3403208|G, 3403225|G, 4262336|G, 3114562|H, 6580983|H, 6580993|H, 4336328|J, 4336329|J, 6093156|K, 6093136|K, 3288388|N, 469239|O, 463057|O, 1732474|CRF01_AE, 1732484|CRF01_AE, 1537050|CRF01_AE, 5931491|CRF01_AE, 1478056|CRF02_AG, 3132800|CRF02_AG, 3132810|CRF02_AG, 6466838|CRF02_AG, 6651465|CRF03_AB, 6651466|CRF03_AB, 5059040|CRF04_cpx, 5059050|CRF04_cpx, 3947925|CRF04_cpx, 5668954|CRF05_DF, 6651454|CRF05_DF, 3779261|CRF06_cpx, 5738565|CRF06_cpx.

I. Samples in the analysis

Of the collected samples, the sequence analysis has been performed on:

- 43 out of 86 samples obtained from the Hospital Bambin Gesù (all for gag analysis)
- 19 out of 121 samples obtained last February at the Centre in Benghazi (5 for gag analysis and 14 for Env analysis)
- 48 out of 98 samples obtained at CIRBA Ivory Coast (all for gag analysis)
Of the performed sequences on gag, this analysis has been focused on the samples coded (code as the number at the Benghazi Centre):

- 406, 136, 356, 300, 379, 153, 265, 310, 34, 139 (samples obtained from the Hospital Bambin Gesù),
- 214, 225, 305, 40, 126 (samples obtained from the Benghazi Centre).

The following samples were analysed from the sequences performed on the samples obtained in Ivory Coast: A65, A66, A68, A69, A80, A71, A83, A28, A36, A57.

2. Subtyping.

All the samples obtained from the cohort of nosocomial HIV-infection at the Al-Fateh Hospital, Benghazi, Libya, were found to belong to the HIV-1 recombinant clade A/G. In fact, the analysis performed with the HIV-1 Subtyping Tool reported for all the sequences the highest homology score for at least 2 different HIV-1 clade A/G reference sequence for each sub-fragment of 100bp submitted to the analysis.

Nine out of 10 samples obtaining from Ivory Coast were also found to belong to the HIV-1 clade A/G. The other one presented a less defined profile that need further investigation on other HIV gene products. This sample (A65) presented a highest homology in the p17 fragment with the recombinant strain A/B, while in the area of p24 the highest homology was obtained for strains belonging to the clade A, G or the recombinant A/G.

3. Virus variability between the groups in analysis.

We first focused our analysis on the overall variability of the gag gene in the group of subjects coming from Ivory Coast in comparison with the group of subjects coming from the cohort of nosocomial HIV-infection at the Al-Fateh Hospital, Benghazi, Libya.

Comparing the HIV-1 gag sequences obtained from the group of subjects from Ivory Coast, not related between them for the source of HIV-infection, 363 out of 7890 bps analysed (4.6%) differed by mutation. This data suggest that the gag sequence variability of clade A/G in a non related population is around 5%. Moreover, 29% (239 position out of 819 bps) of the sequence analysed presented one or more mutations in this group of subjects.

Comparing the HIV-1 gag sequences obtained from the group of subjects from Libya only 103 out of 11835 bps analysed (0.87%) differed by mutation. Furthermore, only 8.5% (67 out of 789bps) of the sequence analysed presented one or more mutations in this group of subjects. Altogether these data suggested that the HIV-1 strains in the Benghazi cohort present a very low
variability confirming that it represents a nosocomial infection coming from one or few related viruses.

Interestingly, when we analysed the samples from subjects that were infected by HIV most probably in the period of 1998 in comparison to the subjects that most probably were infected in 1997 or 1999 (samples 126, 214, 305, 225 and sequence 40 – adult mother of sample 305 -) the variability was still low in both sub-groups (0.76% and 1.08% respectively) suggesting a common origin. Moreover, the phylogenetic analysis suggests a common origin of the strain obtained in both subgroups as they do not clusterise separately.

Similar results were obtained in the analysis performed in Montreal on the lymphocytes of 23 Libyan children hospitalised in Geneva in 1999.

Using nested PCR and primers corresponding to regions of the gag gene and the env gene (V3-to V5 regions), differences of mismatches between the HIV sequences of the children were minimal: for the env region, these differences were in the range of 1.5 to 3.5%. As a comparison, the difference of the children strains with a reference clone of HIV1B, the LAI strain (PLN43) was around 30% for the same region.

All the genetic analyses performed strongly indicate that the nosocomial infection in Benghazi Children Hospital has been caused by a single (or few but very similar) subtype of A/G HIV-1, probably originating from Subsaharan Africa and entered in the Hospital by one (ore few related) HIV infected child who was originally infected by his mother through vertical transmission. This infection was already present in the Benghazi Hospital in April 1997 (the first child sequenced) and was still operating in March 1999 (the last child sequenced).

5. Conclusions

The available data obtained at the Al-Fateh Hospital and the further clinical data obtained by the Centre from Immunodepressed Children (where all children and adults are now followed up in Benghazi) allow to clearly identify the time of the appearance of the HIV infections in the children. According to the Al-Fateh digital List, in the year 1997, at least 7 children were already found infected. At least 14 children admitted and discharged from the Hospital in January and February
1998 (before the Bulgarian staff under Court took in the positions in the Hospital) were found to be seropositive when the analyses were performed in late 1998. According to our direct inquiry at the Centre for Immunodepressed Children, there are few cases suggesting that a low level of vertical transmission and of naturally HIV-infected children was present in the Benghazi area in 1997. This is not surprising, as low level of infection and of vertical transmission has been found in several hospitals which accommodate large number of immigrants from the subsaharan countries. It will be of interest to know whether sentinel testing is performed in the Libyan Hospital to monitor the level of seropositivity in pregnant women.

There is a significant degree of similarity between the HIV strains analysed in the children, according to two scientific publications made by the Swiss and the Italian groups.

Formal proof that the infections occurring in the 1997, then of 1998 and still in 1999 originated from a dominant strain has been proved by direct molecular analysis performed under the supervision of L.M in Montreal and by V.C in Padova. The complete sequences, the number and percentage of mutations are enclosed in the Technical Annex that will be provided the 15th of April.

Several Wards and Divisions of the Al Fateh Hospital (Ward B, C, A, and Isolation-ISO) have been involved in the HIV infections in the year 1997 and then in the first two months of 1998. Other Wards and Divisions were also interested by the infection in the year 1998 and 1999, such A, B, C, G, ICU, ISO, Nephro, Gastro, etc.).

Regarding the modality of transmission, several children are infected also with other bloodborn viruses – HCV (four different clusters) and HBV. The diversity of multiple combinations of viruses strongly support the nosocomial origin of this large outbreak in Al-Fateh Hospital. This has been published by the Swiss group.
STATEMENT
From Prof. Luc Montagnier and Prof. Vittorio Colizzi
on the Benghazi nosocomial infection

- Considering the data provided in digital form by the Al-Fateh Hospital and by the Centre for Immunodepressed Children,

- Considering the clinical checks and the inquiry of parents and medical and nursing staff made at the Centre for Immunodepressed Children,

- Considering the molecular analyses previously performed in Switzerland and Italy and published in international scientific journals,

- Considering the recent molecular analyses performed under our direct supervision in two independent laboratories in Padova (Italy) and Montreal (Canada),

With all these data and information,
with the knowledge of the scientific and medical literatures,

We make the following Statement:

- The HIV nosocomial infection of children which occurred at the Al-Fateh Hospital of Benghazi in 1997-1998-99 has presumably originated from the use of injection material contaminated by blood of one child infected through unidentified horizontal or vertical (more probably) transmission. This putative zero patient was present already in the Hospital before April 1997 (first sequenced child), and the horizontal contamination of some children was already operating in 1997, in the year 1998, and still in March 1999 (last sequenced child). All samples sequenced from these children (1997-1998-1999) belong to a similar viral subtype, strongly indicating a common origin.
• The HIV strain responsible for this nosocomial infection belongs to the subtype A/G, a recombinant form of virus frequent in Central and West Africa. The transmissibility, virulence and pathogenicity of this particular A/G HIV-1 strain has been shown to be very high, as also suggested by the putative retroinfection from some infected children to mothers by breast feeding.

• The high number of cases (around 450), and the period of time of the nosocomial infection (over three years) can be explained by both the high specific infectivity of this strain and certain incorrect practices used by the medical and nursing staff at that time. This assumption is also supported by the high percentage of infected nurses in the Al-Fateh Hospital (two nurses as opposed to a total number of 50 cases of infection in hospital workers all over the world after 20 years of HIV circulation). Alteration of the specific guidelines established to avoid nosocomial infections (not only for HIV but also for HCV), a large introduction of invasive procedures, the shortage of disposable materials leading to the re-use of injection material, are all possible reasons which may explain this massive nosocomial infection.

• No evidence has been found for a deliberated injection of HIV contaminated material (bioterrorism). Epidemiological stratification, according to admission time, of the data on seropositivity and results of molecular analysis are strongly against this possibility.

Prof. Luc Montagnier

Prof. Vittorio Colizzi